

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

First Named Inventor: Ofer Mandelboim et al.

Confirmation No.: 7654

Application No.: 10/562,735

Group Art Unit: 1647

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Examiner: Fozia M. HAMUD

For: FRAGMENTS OF NKP44 AND NKP46 FOR  
TARGETING VIRAL-INFECTED AND  
TUMOR CELLS

Attorney Docket No.: 81589-17600

**DECLARATION UNDER 37 C.F.R. § 1.132**

**Mail Stop : RCE**

Commissioner for Patents

P.O. Box 1450

Alexandria, Virginia 22313-1450

Sir:

I, Angel Porgador, hereby declare as follows:

1. I am a citizen of Israel residing at 11 Tavlan Street, Lehavin 85338, Israel, and am one of the named inventors in the subject application. I wish to submit information in support of the Amendment being submitted concurrently herewith.

2. I am familiar with the contents of the office action. I am an Immunologist by training and experience and have a PhD Degree in the field of Life Sciences from the Feinberg Graduate School, Weizmann Institute in 1994. I am currently employed by the assignee of this application and my current title is Associate Professor. I consider myself to be a person of ordinary skill in the field of the present invention.

3. I understand that claims 1-11, 20-27 and 47-48 have been rejected for allegedly failing to satisfy the enablement requirement. To provide further support for the claims, by me or under my direction and control experiments were conducted with three NKp46 D2 domain peptides, designated as Peptide #7, Peptide #12 and Peptide #13. The NKp46 D2 domain corresponds to amino acid residues 121-254 of intact NKp46 isoform a and has the following amino acid sequence: YDTPT LSVHP GPEVI SGEKV TFYCR LDTAT SMFLL LKEGR

SSHVQ RGYGK VQAEF PLGPV TTAHR GTYRC FGSYN NHAWS FPSEP VKLLV TGDIE NTSLA PEDPT FPADT WGTYL LTTET GLQKD HALWD HTAQ. Each of Peptide #7, Peptide #12 and Peptide #13 is 20 amino acids in length. These peptides were tested for (i) direct binding to target cells; and (ii) their effect on the binding of the fusion protein NKp46D2-Ig to human cancer cells (HeLa), MIN6 murine beta cell lines and primary mouse beta cells.

4. The experiments were specifically conducted as follows. First, target cells were incubated for 1.5hr with 2µg/well NKp46D2-Ig and 10µg/well of each peptide. NKp46D2-Ig contains the second NKp46 domain and the membrane linker peptide, which is fused to the Ig (CH2+CH3) of human IgG1. Then, NKp46D2-Ig binding intensity was determined by flow cytometry, using fluorescent 2Ab (gout anti-mouse mAb). Afterwards, Biotin-conjugated peptides were employed separately (10µg/well at 100 µl) to assess direct binding of the peptides to target cells using fluorescent Streptavidin as the second step.

5. As shown in **Table 1**, the results show that each of Peptide #7, Peptide # 12 and Peptide #13 showed direct binding to target HeLa cells. Moreover, the experimental results also showed that each of Peptide #7, Peptide # 12 and Peptide #13 enhanced NKp46D2-Ig binding to target cell population, as compared to NKp46D2-Ig staining without peptide or with non-affecting peptides. As a negative control, the results further revealed that the binding of another recombinant NK receptor, LIR1-Ig, was not affected in the presence of anyone of the NKp46 affecting peptides, i.e., Peptide #7, Peptide #12 and Peptide #13.

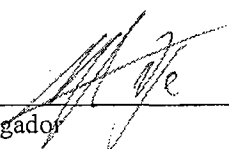
**Table 1.** NKp46D2-derived peptides with effect

| Peptide | Sequence                | Direct binding to HeLa cells | Effect on NKp46D2-Ig binding to HeLa cells |
|---------|-------------------------|------------------------------|--|
| #7      | SMFLL LKEGR SSHVQ RGYGK | Yes (1)                      | Enhance (2)                                |
| #12     | PLGPV TTAHR GTYRC FGSYN | Yes (3)                      | Enhance (3)                                |
| #13     | TTAHR GTYRC FGSYN NHAWS | Yes (2)                      | Enhance (1.5)                              |

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6. I hereby declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Signed this 28 day of March, 2010.

  
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Angel Porgador

**Prof. Angel Porgador**  
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